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Improving Germination of Tomato Seed by Electric Field Treatment

Mamta Samir Patwardhan[1], Waman Ziblaji Gandhare [2]

1 Department of Farm Structures and Rural Electrification, Dr. A. S. College of Agricultural Engineering, Mahatma Phule Agricultural University
patmam@rediffmail.com

2 Government College of Engineering, Amravati
wz_gandhare@yahoo.co.in

ABSTRACT:

This study was conducted to determine the effects of Electric field treatment on tomato seed germination. The experiments were conducted to study the behavior of seed after exposure of electric field in different manner such as Electrostatic, Microwave. It was found that its germination was sensitive to electric field applied. For electrostatic field exposure of 20 kV/cm for 20 second duration was found as a best treatment. For microwave treatment the power level of 70%, 630 W for 20 sec duration was found best. The statistical analysis was carried out to confirm the results.

Keywords: Tomato Germination, Microwave, Enzymes, Microorganizms

INTRODUCTION

In 2000, the world population exceeded six billions. However farmlands are reducing because of the soil deterioration, water shortage and civilization *etc*. Thus, world probably falls into a serious food scarcity. Hence there are requirements of bioresearch to improve the harvest efficiency of food plant. The various investigations have been carried out [1]. They were termed under the technique of 'Electro-culture'. The several approaches to Electro-culture include: antennas, static electricity, direct and alternating current, magnetism, radio frequencies, monochrome and intermittent lighting and sound. The energies are applied to the seeds, plants, soil or the water and nutrients [2].

Plant growth as well as the biological processes of seeds, can be accelerated or inhibited by high intensity electric fields. The mechanism of these actions is still insufficiently known.

Destruction of microorganizms in liquids by using high intensity electric fields has been thoroughly investigated by many scientists. A review of the efforts on the inactivation of microorganisms by pulsed electric fields can be found in [3]. The electric fields effects were mainly attributed to the field induced intensification of the biological processes in seeds. Positively or negatively ionized air induced more rapid germination of seeds.

Several approaches [3–8] to electro-culture include: antennas, static electricity, direct and alternating current, magnetism, radio frequencies, monochrome and intermittent lighting and sound. The energies are applied to seed, plants, soil or the water and nutrients. Electrostatic treatment is assumed to enhance seed vigor by influencing the biochemical processes, which involve free radicals and by stimulating the activity of proteins and enzymes [9]. Corona discharges also seem to affect the biological activity of seeds [10]. Many researchers demonstrated positive effects and negative influences on seeds by magnetic field.

Hence, there is a strong need to assess all possibilities to improve agricultural production. An attempt has to be made to increase the crop yield by using advanced techniques of agriculture. Accordingly, the study based on electro-culture technology to enhance seed germination; was undertaken at Mahatma Phule Agricultural University, Rahuri. This paper discusses about electric field application for tomato seed germination improvement.

EXPERIMENTAL PROCEDURE

Tomato is one of the important cash crops in the world. It is a high value seed crop. Production using transplanted seedlings of vegetables crops is an important method for vegetable growers. Healthy seedlings ensure the successful development of the plants and ultimately result into more profits for the farmer. Moreover, in a climate controlled greenhouse, healthy seedlings can be produced in advance as compared to those produced in open field conditions.

The classical methods of seed treatment make use of chemical substances which are either expensive or harmful to the soil. The data presented in this paper demonstrates that exposure to a high –intensity electric field can be an effective substitute for germination improvement.

The trials were undertaken on old tomato seeds of 2007, 2008 and 2009 year batch. Seeds of tomato *cv.* Dhanashree developed by Mahatma Phule Agricultural University were used for trials.

Germination tests were conducted by applying electrostatic field of different intensity as well as interval for a lot containing 50 seeds /lot with three replications. Similarly microwave field was used to treat tomato seeds.

The germination trials were conducted in two phases i.e. germination at pot trials and seed germinator. For seed germinator petri dishes and for open field plug trays were used.

The student t- test was used for analyzing the correlation between the electric field exposure and seed germination, root shoot length etc. The computed values for each test experiment were compared with those given in tables for different confidence level α. The significance of a correlation was evaluated in function of the value of α. For instance, $\alpha < 0.05$ means that the investigated effect of electrostatic field is considered significant.

For applying electrostatic field the experimental set up was arranged at Electrical Engineering laboratory. It included HV transformer, controller unit, electrodes, HDPF film etc. The detailed arrangement was shown in Figure 1.

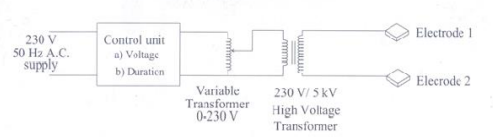

Figure 1: Schematic Arrangement for Test Set up

The seeds were kept between electrodes covered with HDPE film to avoid the contact with each other. This will add a capacitive effect hence electrostatic field will be developed.

LABORATORY TESTS

Several laboratory experiments were performed to determine whether high – intensity electric field exposure improves germination. Each test involved several exposures of a electrostatic field. The influence of microwave irradiation on seeds of onion, soybean and tomato has been investigated. A microwave; operating on 230V, 50 Hz, single phase supply, 900W maximum power output with operating frequency 2450MHz was used to give treatment as a source.

The tomato seeds for the experiment were distributed in nine lots each containing 50 seeds with three replications. The variants differ by the time of exposure to electrostatic and microwave radiation. Seeds were exposed to the duration for each method 0 s (control), 10 s, 20 s, 30 s. Electrostatic field with potential gradient as 10 kV/cm, 20 kV/cm and 30 kV/cm and Microwave radiation with output powers of magnetron – 80%, 70% and 60% were applied.

Group of 50 seeds were subjected to each each treatment, for chosen exposure time and analogous groups were used as control. Then tomato seeds were cultured in petri dishes for germination trials. No ohmic heating effect was observed. The laboratory tests were conducted with the help of following equipments.

1. High voltage test set up, Microwave
2. Meters for voltage and current measurements as well as waveform storage facilities.
3. Seed germinator having humidity, temperature and light intensity control facility.
4. Oven for dry matter measurement, weighing balance, seed counter, electrical conductivity meter.
5. Miscellaneous material required for actual germination trials such as germination paper, wax paper, petri dishes, blotting paper, cotton, chemicals etc.

In all, there were ten samples along with one untreated as a control. The germination tests were carried out in seed germinator where temperature and humidity was maintained at 25^0 at 80% relative humidity. Similarly same seeds were sown in the plugs filled with soil having sufficient moisture well suited as open field conditions. In order to estimate the influence of the microwave treatment on tomato seed following factors were considered:-

Germination percentage
Root- shoots length of 10 seedlings
Fresh weight of 10 seedlings
Dry weight of 10 seedlings

The trials were conducted for seed lots of different years. Fresh seed lot of 2012 was used. Old seed lots from year 2007 to 2009 were considered. For fresh seed lot 50 seeds/lot with three replications and for old seed 400 seeds /lot were tested for germination trials at germinator and pot respectively.

Additionally the electric field was applied by different techniques such as corona discharge test set up, magnetic field set up. In each case the enhancement in seed germination was observed.

RESULTS AND DISCUSSIONS

After the treatment seeds were used for germination trials immediately. The germination percentage were counted as first day count and final count on fourth and fourteenth day as per ISTA standards. Percentage germination, root – shoot length, fresh weight, dry matter weight, seed vigour1 and seed vigour2 were calculated from test results. Table 1 gives the results of germination percentage for the trials conducted.

The microwave treatment was given for tomato seeds. The observations were noted at fourth day and tenth day. Treatment P2T2 method (Power level 70% , 20 sec time duration) showed better

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results from germination, seed vigour point of view. It was found that irrespective of seed lot year microwave impact was observed for almost all power levels as well as time of application. Out of that the method of P2T2 was consistently proved better.

Table2 shows that the method V2T2 (2 kV/mm, 20 sec) is most efficient method. Thus it was observed that both electric field application methods were effective to enhance the seed germination. This was the important aspect from farmers' point of view as tomato seeds are too costly. If they use the untreated seed, their production will be hampered due to less germination. It reveals that excess seeds may be in use rather than wastage; farmers get benefit by earning good returns from seed/ seedlings/ crop.

Further this germination trials results were cross checked by doing bio chemical analysis. The enzyme activity study was done.

i. Lipid peroxidation: The assay of enzyme activity and lipid peroxidation was carried out within seven days after seeds were exposed to electrostatic field as shown in table 3. Three replications of 50 seeds/treatment were allowed to imbibe for 10 h, then hand homogenized in an iced mortar with pestle in 4ml of 0.1M potassium phosphate buffer(pH 7.0), followed by centrifuging at 10,000 g for 20 min, the supernatant obtained was used for determining enzyme activity and total soluble protein.

ii. SOD activity: Superoxide dismutase (SOD) activity was assayed using photochemical method as shown in table 3. Every 1ml of the reaction mixture contained 0 -200 µl enzyme extract, 1.3µM riboflavin, 13 mM methionine, 63 µM nitroblue tetrazoliun (NBT), 50mM Na-phosphate buffer (pH 7.8) and 0.1mM EDTA. The test tubes containing the reaction mixture were shaken and placed 30 cm below a light blank consisting of two 15 w fluorescent tubes. The reaction was allowed to run for 10 min, and then stopped by covering the tubes with a piece of black cloth. The reaction in NBT was followed by reading the absorbance at 560nm. The non-irradiated reaction mixtures were used as the blank. One unit of SOD activity was defined as the amount of enzyme causing 50% inhibition to the initial reaction rate (the rate of reaction in absence of the enzyme).

Table 1: Germination Percentages for Microwave

	2007	2008	2009
P1T1	13	19.75	47
P1T2	13.75	20.5	25
P1T3	15	23	29
P2T1	15.75	23.5	28
P2T2	16.25	24.5	50
P2T3	14	21.75	28
P3T1	14.5	24	23
P3T2	13	22	29
P3T3	12.25	18	23
C	10	16.25	21

Table 2: Germination for Electrostatic Field

Treatment	Treatment details	% Germination 2010
Control		92
V1T1	1kV/mm, 10sec	98.7
V1T2	1kV/mm, 20sec	98.7
V1T3	1kV/mm, 30sec	98
V2T1	2kV/mm, 10sec	98.7
V2T2	2kV/mm, 20sec	**100**
V2T3	2kV/mm, 30sec	98.7
V3T1	3kV/mm, 10sec	98
V3T2	3kV/mm, 20sec	98.7
V3T3	3kV/mm, 30sec	99

Table 3: SOD Activity Calculations

Treatment	OD at 560		Difference	SOD activity (unitg^{-1}FW)
	Light	Dark		
V_1T_1	0.337	0.032	0.305	3.078
V_1T_2	0.335	0.011	0.324	3.10259
V_1T_3	0.414	0.013	0.401	4.6525
V_2T_1	0.284	0.030	0.254	4.6106
V_2T_2	0.980	0.212	0.768	6.8052
V_2T_3	1.120	0.271	0.849	4.8306
V_3T_1	0.666	0.156	0.510	4.7016
V_3T_2	0.875	0.235	0.640	4.1053
V_3T_3	1.950	0.560	1.39	7.854
C	0.492	0.309	0.183	1.5279

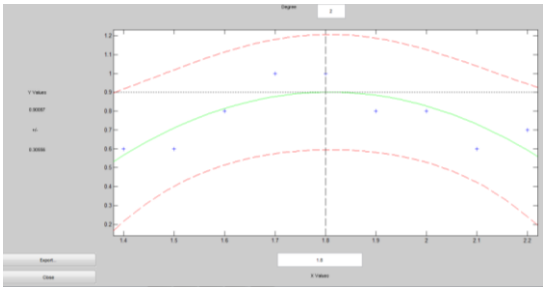

Figure 2: Graph Showing Optimal Point of High Voltage

Data analysis was performed using Excel 2007 as well as Minitab and MATLAB2010. Two way ANOVA was calculated for each parameter. Curvilinear regression analysis was also adopted for representing germination as a dependent variable in equation form using voltage applied. It can be seen that the local optimum point is found from following formula:- (Where x is the voltage applied and y is germination percentage)

$$y = 75.33 + 18.76\,x - 5.53\,x^2$$

$$x = (-b_1 / 2b_2)$$

means at 1.69 kV , maximum germination takes place.

To achieve optimum voltage and duration another trials were conducted with precise changes in voltage ranging from 1.4 kV to 2.2 kV with an incremental step of 0.1 kV. It was found that 1.8 kV at 20 sec. duration gave best practical results. For time optimization another test was conducted with 1.8 kV, 10 to 50 sec. duration with the incremental duration of 10 sec., it was reconfirmed that 1.8 kV, 20 sec. was the best option.

```
Y=[0.6 0.6 0.8 1 1 0.8 0.8 0.6 0.7]
V=[1.4 1.5 1.6 1.7 1.8 1.9 2 2.1 2.2]
polytool(V,Y,2)
Using minitab:
MTB > Regress 'y' 2 'v' 'v2';
SUBC>GNormalplot;
SUBC>GFits;
SUBC>RType 1;
SUBC>  Constant;
SUBC>  Brief 2.
```

Regression Analysis: y versus v, v2

The regression equation is

y = - 5.68 + 7.28 v - 2.01 v2

Predictor	Coef	SE Coef	T	P
Constant	-5.681	2.032	-2.80	0.031
v	7.280	2.291	3.18	0.019
v2	-2.0130	0.6351	-3.17	0.019

S = 0.1115 R-Sq = 62.7% R-Sq(adj) = 50.3%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	2	0.12547	0.06274	5.05	0.052
Residual Error	6	0.07453	0.01242		
Total	8	0.20000			

From the regression equation the optimal value of v is,

v= 7.28/4.02 =1.810 kV

Using Matlab:

Application

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```
Y=[0.6 0.6 0.8 1 1 0.8 0.8 0.6 0.7]
V=[1.4 1.5 1.6 1.7 1.8 1.9 2 2.1 2.2]
   polytool(V,Y,2)
```

By using this equation the decision for best germination voltage gradient applied can be taken while applying electrostatic field for tomato seeds.

Seed treatment has many methods. According to [8], electrostatic field is better to pre-treat thin coat seed. The germination enhancement is due to electrical nature with potential differences existing in all tissue cells. Polarization phenomenon occurs in the seed tissue. Therefore, an inner electric field induced by external electric field would form in the seed, and its magnitude will depend on the magnitude of the external electric field. The seed cell membranes are repaired due to electric conductivity change; this modifies respiratory intensity and dehydrogenises activity of seed and the hydrolysis of fat accelerates. β – oxidation of seeds speeded up, the supply of sugar ensures for seed respiration and germination as well as seed vigor.

Destruction of microorganisms in liquids by using high intensity electric fields has been thoroughly investigated by many scientists. A review of the efforts on the inactivation of microorganisms by pulsed electric fields can be found in [7]. The electric fields effects were mainly attributed to the field induced intensification of the biological processes in seeds. Seed deterioration concerned with free radicals. The highly aggressive free radicals produced by auto-oxidation in dry seed can react with majority of bio-molecules, causing cellular damage. Success of germination could largely depend upon the activity of anti - oxidative systems to prevent cellular components from being damaged by free radicals.

The polarization effect of high voltage electric field upon dielectric substance can cause the hydrogen bonding in water to break or bend, hence it is reasonable to assume that during the electric field exposure of seed could also bend or break the hydrogen bonding in ultra - structural elements of the cell, e.g. enzymes, resulting in structural alteration of the micro-molecules. This may increase enzyme activity; or cause enzyme denaturizing, depending on the strength on electric field and time of exposure. Furthermore, free radicals and metal ions bearing some kind of electrical charge might move towards electrodes, result in radical – radical recombination and replacement of metal ions. Hence free radical level is decreased and the cell balance is improved.

Accordingly the electrostatic field enhances the process of germination. This technology will be very much important for rural agriculture for sustainable development. As this method is very simple for adoption, its commercialization is possible. This will be a great engineering tool for seed users to improve the seed storage life and quality with best efficiency.

Thus with adoption of electric field for seed treatment, the germination and seed vigour can be improved. Ultimately it results in maximum yield. The food production may be increased. As per review, this technique results in reduced fertilizer and pesticide requirement; that will be an added advantage. This will help rural development and create tremendous wealth in these areas. But still technology needs to go a long way in the process of research and development so that it can be made available at economical rates and the feasibility can be increased.

With reference to national policy for farmers, there is a need to focus more on the economic well- being of the farmers, rather than just on production. This study will be beneficial for farmers for gaining benefits from older seeds. By carrying such research, using electricity in optimized way, productivity on farm can be improved to meet increased food demand of huge population.

CONCLUSIONS

1. The method of electric field exposure for tomato seed was found as an effective tool for older seed germination enhancement
2. On comparison of traditional practice, electro statically treated tomato seeds were shown statistically significant results for improvement in germination percentage and subsequent seed vigour
3. The germination tests proved with statistically significant results when electrically treated samples are compared with untreated ones
4. Tomato seed emergence was found optimal based on statistical analysis for electrostatic field with voltage gradient of 1.8 kV/mm, with 20 second duration
5. Treatment P2T2 method (Power level 70% , 20 sec time duration) showed better results with two fold increment from germination, seed vigour point of view

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